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A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*

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The systematic sequencing of the yeast *S.cerevisiae* genome has revealed a profusion of Open Reading Frames (ORFs). Although some of them have been previously studied, a large majority represents new genes (1, 2). Deletion of these ORFs is a convenient tool for their functional analysis, Here we describe a new approach for generating null alleles of a gene. Usually, a gene inactivation requires the *in vitro* creation of a construction in which a selectable marker is sandwiched by the 5′ and the 3′ flanking sequences of the target ORF. Classically, this strategy requires several cloning steps. In contrast, our approach generates such a construction by one step PCR amplification. Each oligodeoxynucleotide used contains two distinct regions, one which allows homologous recombination at the target locus and will be named the deleting sequence, the second part which permits the PCR amplification of a selectable marker. The deleting sequences, which are respectively the 5′ (oligopro) and 3′ (oligoterm) flanking sequences of the ORF, range from 35 to 51 nucleotides in length and are followed by a short stretch of 17 nucleotides homologous to the *HIS3* selectable marker. Table 1 shows the composition of the deleting sequences, the sequence used for *HIS3* amplification always being the same (5′-TCGTTCAGAATGACACG-3′ for oligoterm and 5′-CTCTTGGCCTCCTCTAG-3′ for oligopro.

Following the PCR amplification, the crude mix was directly used to transform yeast by standard procedures (3). In a first set of experiments, we used W303-1B (*MAT α, ura3-1, trp1-1, ade2-1, leu2-3, 112, his3-11, 15*) as recipient strain. All the His$^+$ transformants tested (a total of 30) presented the same pattern when analysed by Southern blot, corresponding to the insertion of the wild-type *HIS3* gene at its own locus (data not shown). We thus used a recipient strain carrying a complete deletion of the *HIS3* gene. With the diploid strain BMA 1 (a diploid from cross FY 1679-18B and FY 1679-28C, kindly provided by B.Dujon) containing the His3Δ200 allele (4), we routinely obtained more than 10 His$^+$ transformants per plate. As the procedure appeared to be efficient enough, we tested by

Table 1.

Tested ORF	Transformants with the expected deletion among His$^+$ clones	'Deleting sequence' of the oligodeoxynucleotides used in this study
YCL09C	3/5	5′-CCCAGTCTTTCTAATGCTGAGATCGTTATTGCAAAGCGGCCACCGCAGGG-3′ Oligopro 5′-GGGCCGAGAGACTAACCTAACCAGGTGGTAGTTGGGAAATGTCGACCA-3′ Oligoterm
YCL11C	4/5	5′-AGATCACCTGTACGTCGTCGTTTGAGCGACGACAG-3′ Oligopro 5′-ACAGCAACACCAGTTGGTTGACCATTTTCCTGTGG-3′ Oligoterm
YCL14W	1/6	5′-GTATATTTACACCTCACCAATGGAGAAAGACCTGTCGTCTCTTTACTCTG-3′ Oligopro 5′CATCTGCAAAATTGGAATGTTATCCTGCTTTGTGGATTGATCCGCTTTTG-3′ Oligoterm
YCR81W	2/5	5′GCTGGGGCAGATGATGCCATTTCCATGCACCTGCTAAAGGACTGGAC-3′ Oligopro 5′-CCATTTGGATATATTCTGAGATCAATGTGGATTTTTCCTCTCAAACCGAGC-3′ Oligoterm

The plasmid used as matrix was constructed as follows: a 1.7 kb BamHI–BamHI fragment including the *S.cerevisiae HIS3* gene was cloned into pUC 18 at the BamHI site. PCR amplification was performed in a total volume of 50 μl containing 0.5 μg of the plasmid described above, 200 μM of each dNTP, 5 μl of 10×buffer (500 mM KCl; 100 mM Tris–HCl pH 8.8; 15 mM $MgCl_2$; 1% Triton X100; 0.1% gelatin), 0.5 μM of each primer (oligoterm and oligopro) and 2.5 units of Taq polymerase (Bioprobe). The reaction mix was incubated for 5 minutes at 95°C and submitted to 2 cycles of PCR (30 secs at 94°C, 30 secs at 45°C and 2 min at 74°C) followed by 30 cycles (30 secs at 94°C, 30 secs at 50°C and 2 min at 74°C). In the final cycle, the extension step was for 5 min. 1 μg of the PCR product (1100 bp long) was used directly to transform yeast cells, For each ORF, rransformants were analysed by a Southern blot using the HIS3 gene as probe.

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Southern blot a maximum of 6 clones for a deletion assay. Table 1 summarizes the results obtained with 4 different deleted ORFs. Among this limited set of data, we did not observe any correlation between the length of the deleting sequence (oligopro or oligoterm) and the efficiency of the method. After sporulation, the presence of His^+ and His^- spores was verified in the progeny of the correctly deleted clones, indicating a non-esssential role for the targeted genes and a normal segregation of the marker.

Our method appears to be particularly convenient for the functional analysis of the ORFs deduced from the systematic sequencing of the yeast *S.cerevisiae* genome, since only the knowledge of the sequence is required for attempting a deletion. As the PCR amplification is the sole molecular biological step, the gene inactivation is thus greatly facilitated. We now plan to prepare suitable deletions for other auxotrophic markers in order to increase the range of application of this technique and notably to allow multiple deletions in the same strain.

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